

## REITER PROTEIN LATEX TEST

### A PRELIMINARY REPORT\*

BY

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A large number of simple flocculation tests with lipid extracts are available for the diagnosis of treponemal infections, but little has been published on such techniques using treponemal extract antigens. It seemed worthwhile to investigate whether the widely used latex agglutination technique could be applied to detect treponemal antibodies in place of the more elaborate complement-fixation methods.

In the present paper a procedure, similar to that, reported previously for hydatid disease (Fischman 1960a), using polystyrene latex particles coated with Reiter protein antigen is described, and the results are compared with the Reiter protein complement-fixation test.

#### Methods

##### REITER PROTEIN LATEX TEST (RPL)

###### Reagents

**Latex.**—Bacto Latex 0·81, as supplied by Difco, Detroit, USA. This is a polystyrene latex suspension diluted from stock Dow Latex. Bacto Latex when diluted further 1 : 100, gives 5 per cent. transmission on a Coleman Junior Spectrophotometer at 650 m $\mu$ . If original Dow Latex is used in place of Difco Bacto Latex, this should be diluted with water to produce a suspension of the same density as Bacto Latex.

**Stock Antigen.**—Reiter Protein Antigens: Sylvana Co., Millburn, USA (Lot 54, RPCF labelled titre 1 : 60) and Sclavo, Siena, Italy (No. 2E3, RPCF labelled titre 1 : 20).

**Buffer.**—975 ml. 0·1 M glycine and 2·5 ml. 1 N NaOH were made up to 1,000 ml. distilled water and the pH adjusted to 8·2; 10 g. NaCl were added.

###### Procedure

(1) Sera were inactivated for 30 min. at 56°C. If not tested the same day, they were kept frozen at -20°C.

(2) Antigen suspension (sufficient for twenty tubes) was prepared by adding 0·15 ml. latex to 10 ml. buffer in a

10×3 cm. tube and the suspension mixed by swirling. Then 0·2 ml. Stock Antigen (Sclavo), or 0·1 ml. Stock Antigen (Sylvana) was added and the suspension mixed by swirling twenty times. It was allowed to stand for at least 15 min. before use.

(3) 0·5 ml. buffer were added to three 10×1 cm. tubes. 0·5 ml. serum was added to the first tube, and doubling dilutions made in 0·5 ml. volumes. Dilutions 1 : 2 and 1 : 4 were tested; the third dilution was kept frozen for completing titration later if desired. A suitable one-tube positive control was also tested with each antigen suspension.

(4) 0·5 ml. antigen suspension was added to each tube. The tubes were shaken and incubated at 37°C. for 90 min. This was followed by refrigeration at 4°C. overnight.

(5) Next day the tubes were centrifuged in an MSE "major" centrifuge for 3 min. at 2,300 r.p.m.

###### Reading:

Deposits were resuspended by light tapping of the tubes and observed with the naked eye. Large floccules were visible in a strongly positive, small floccules in a weakly positive test. Negative tests were turbid with no macroscopically visible floccules. Reading of deposits only, without resuspension, is unreliable, as sediments are often seen after centrifuging in negative tests also. Inspection of settled granules 30 min. after tapping helps to clarify doubtful results.

#### OTHER TESTS

**Reiter Protein Complement Fixation (RPCF) Test.**—Doubling dilutions of sera (1 : 4–1 : 8 for routine, 1 : 2–1 : 128 for full titre) were tested with Sylvana Antigen 1 : 60, using five 50 per cent. haemolytic units of complement (Kent, Covert, Reilly, Kinch, and Lawson, 1962).

**Routine Tests (STS).**—Wassermann reaction, using crude heart extract in the Richardson-Wyler technique adapted to 50 per cent. haemolytic units (Whillans, 1950), and a cardiolipin flocculation test with "Wellcome" antigen.

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## Results

Both RPCF and RPL tests were performed on the following sera:

(A) **Presumed Healthy Donors with Negative STS (120).**—All sera were negative with both tests, except three. These three were reactive in the RPL in a dilution of 1 : 2 only. One of these also reacted in the RPCF, the other two were non-reactive.

(B) **Specimens selected from Sera received for Routine Testing (268).**—

(a) All sera with positive STS.

(b) Sera with negative STS, if treponemal test requested by physician.

There are no facilities for the TPI test in New Zealand.

The results were divided into three groups:

(1) **Latent Treponemal Infection (125).**—Patients were coloured Pacific Islanders with a history of yaws or came from an island in which yaws was endemic. The results are shown in Table I.

TABLE I  
COMPARISON OF RPL AND RPCF TESTS ON SERA OF PATIENTS FROM AREAS OF ENDEMIC YAWS

Result	Tests	No.	Per cent.
Agreement ..	Both+	81	87.2
	Both—	28	
Disagreement ..	RPCF+ RPL—	6	12.8
	RPCF— RPL+	10	

The overall sensitivity of the RPCF and RPL was similar. 12.8 per cent. of results showed disagreement, only one of the tests being reactive. RPL reactivity was very slightly higher than RPCF reactivity (72.8 and 69.6 per cent. respectively). This is not statistically significant.

(2) **Syphilitic Sera (69).**—RPCF and RPL showed agreement in the majority of cases, in both the early and the late groups, the closest agreement being in the late untreated series. Disagreement was found in 13 per cent., in which the sera reacted with RPL or RPCF alone. In the early untreated group, the RPCF detected one case more than the RPL (Table II).

(3) **Miscellaneous Group (74).**—Clinical diagnoses were not available in these patients. Of these, 25 sera were reactive, and 43 were non-reactive with both tests. The RPCF was positive also in two, and the RPL positive alone in four sera.

TABLE II  
COMPARISON OF TESTS ON SYPHILITIC SERA

Result	Tests	Syphilis				Per cent.
		Early		Late		
		Un-treated	Treated	Un-treated	Treated	
Agreement	Both +	12	9	13	12	87.0
	Both -	3	6	1	4	
Dis-agreement	RPCF + RPL -	2	1	0	1	13.0
	RPCF - RPL +	1	2	0	2	

**ANTIGEN STRENGTH AND COMPARATIVE TITRES.**—Titres of RPL and RPCF frequently showed lack of correlation, lower titres in one test being accompanied by higher ones in the other. Good agreement was shown for the two standard sera used. The Sylvana positive control was reactive at a titre of 1 : 64 in both procedures. The pooled standard of this laboratory was reactive at a titre of 1 : 16, and non-reactive at 1 : 32 in both RPL and RPCF.

While two lots of Sylvana antigen gave these satisfactory sensitivities, a third lot was found to be of low sensitivity for the RPL. An increase in the amount of stock antigen used per antigen suspension did not improve the results. One lot of Sclavo antigen tested gave satisfactory sensitivity, while one batch of Organon antigen failed to produce acceptable sensitization of latex (Table III).

TABLE III  
EFFECT OF VARIOUS BRANDS AND AMOUNTS OF ANTIGEN ON RPL TITRE

Antigen	ml.	Titres of Four Sera			
Sclavo No. NE3 ..	0.2-0.3	32	4	32	8
Sylvana Lot 54 ..	0.05	32	0	16	8
Sylvana Lot 54 ..	0.1	64	4	64	16
Sylvana Lot 54 ..	0.15	64	8	128	16
Sylvana Lot 59 ..	0.1-0.2	8	0	16	4
Organon 442715 ..	0.2-0.3	4	0	4	0

No further batches have been evaluated so far. No difference in sensitivity was noticeable with any of these antigens in the RPCF.

## Discussion

Results indicate that latex may be coated with Reiter protein antigen and that the coated particles are suitable to demonstrate the presence of treponemal antibodies. The RPL technique has a sensitivity

and specificity comparable to RPCF. In 13 per cent. of treponemal infections only one test or the other was reactive. Thus the tests are not completely interchangeable. Further work on a larger series and reference to TPI or FTA results is needed for more reliable evidence whether the RPL is suitable for routine use.

Latex techniques are simpler than complement-fixation procedures, requiring less standardized reagents. Even more rapidity may be obtained with latex slides, using mechanical shaking (Fischman, 1960b) or tilting by hand. Preliminary slide experiments with Reiter protein (unpublished) have shown less satisfactory results as regards sensitivity and specificity than the tube technique.

A limitation of the use of the RPL test is the considerable variation in sensitivity shown by latex suspensions coated with different antigens. Even with the same brand of antigen, two highly sensitive and one insensitive lot were encountered. Of two other commercial antigens only one gave satisfactory results in the RPL. Suitability for the RPCF was not affected. No explanation was found for this phenomenon. Discrepancy may be due to different methods of preparation, or to added substances making up the final commercial product. Cannefax (1963) has shown that different lots of Reiter protein antigen with the same RPCF titre show great variation in their serologically active protein and carbohydrate content. This may have some bearing on the varying uptake of antigenic components by latex.

Further evaluation is needed as regards significant titre. With antigens of high sensitivity good separation of positives and negatives may be achieved by accepting only titres of 1 : 4 or more as reactive. With other antigens this cannot be done without loss of sensitivity, and some overlapping may occur in the 1 : 2 dilution.

The latex technique may provide simple procedures for testing with treponemal extracts other than Reiter protein.

#### Summary

- (1) An agglutination test using polystyrene latex particles coated with Reiter protein for the

detection of treponemal antibodies is described.

- (2) A preliminary evaluation of sera from presumed normal persons and from patients with treponemal infection shows close parallelism between this test and the Reiter protein complement-fixation test as regards both sensitivity and specificity. Results agreed in 92 per cent. of all sera. The latex technique is simpler than the complement-fixation method.
- (3) Reiter protein antigens vary in their suitability as sensitizers of latex. This property seems independent of the potency of an antigen for the RPCF test. Only some selected antigens give sufficient sensitivity in the RPL test.

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#### Le test d'agglutination du latex de la protéine de Reiter (RPL)

##### RÉSUMÉ

(1) On décrit une méthode qui permet d'identifier les anticorps tréponémaux par les molécules de latex polystyrène couvertes de protéine de Reiter.

(2) Une évaluation préliminaire des sérums normaux et de ceux des malades atteints d'infection tréponémale indique que ce test ressemble à celui de la fixation de complément par la protéine de Reiter (RPCF) en ce qui concerne la sensibilité et la spécificité. Les résultats sont semblables dans 92% des sérums. Cependant, la méthode du test RPL est plus simple que celle du test RPCF.

(3) La sensibilité des antigènes est variable, et semble indépendante de celle du test RPCF. Seuls quelques antigènes choisis ont assez de sensibilité pour le test RPL.